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Cell Motility and Guidance

Program/Abstract # 189
SMN participates in the positioning of motor neurons in the ventral neural tube

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Motor neurons extend their axons ventrally to innervate specific muscles in the hindlimb. We are examining the role of SMN, the gene that is responsible for spinal muscular atrophy (SMA), using a loss-of-function approach. First, we assessed whether SMN levels were reduced using SMN shRNAs in vitro in HEK293T cells; SMN levels were indeed decreased by SMN shRNAs but not by missense shRNAs. In vivo, at early stages when SMN levels are reduced, motor axons are growth-impaired and exhibit likely fasciculation defects. Surprisingly, when SMN levels are reduced in vivo, Islet-1-positive motor neurons appear to migrate aberrantly out of the neural tube, along the spinal nerve at later stages; this was never observed in control electroporations. We have examined the patterning of cells in the neural tube and find that it was mispatterned. Interestingly, boundary cap (BC) cells were in place, as shown by various markers (cadherin7, lingo-1 antibodies). Semaphorin6A was normally expressed by BC cells; when SMN was reduced in motor neurons, Semaphorin6A altered its expression to motor neurons. To determine if Semaphorin6A was responsible for the escape of neurons from the neural tube, Semaphorin6A was expressed by motor neurons using in ovo electroporation. Islet-1 antibody staining revealed that Semaphorin6A could account for early stages of escape of motor neurons in SMN-reduced embryos, but not at later stages. Experiments are underway to determine exactly why motor neurons leave the neural tube when SMN levels are decreased.

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Program/Abstract # 190
Going rogue: In vivo analysis of axon transport in zebrafish

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In neurons, proteins and organelles must be transported to specific compartments to form functional synapses and maintain cell health. Studies *in vitro*, complimented by invertebrate work, give insights into the basic mechanisms of this process. However, to date, a vertebrate system in which to analyze axon transport in real time in normal and diseased states had not been developed. We established the zebrafish as a system for studying axon transport *in vivo* by first developing an imaging approach to visualize fluorescently labeled cargo using *in vivo* time-lapse microscopy. Using this novel approach, we show that transport velocity differs according to the developmental stage of the neuron. Second, using forward genetics, we have identified a set of recessive zebrafish mutant strains with defects in axon transport. One of these strains (*rogue*) displays truncation of long sensory axons and swollen axon terminals. We have identified the gene mutated in *rogue* as *jnk interacting protein 3* (*jip3*). Jip3 has been implicated in axon transport and axon extension in other systems but the cellular mechanisms it mediates are not well defined. Transport velocity of certain cargo is dynamically altered in *rogue* and both distance from the cell body and developmental stage influence these changes. We are continuing to use the *rogue* mutant to define the role of Jip3 in axon transport and to determine how deficits in this process result in failed axon extension. Introduction of this new vertebrate system will provide more insight into the mechanisms of axon transport and how alterations impair development and maintenance of neural circuits.

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Program/Abstract # 191
Distinct mechanisms of Slit-Robo attraction and repulsion mediate the guidance of glial cell positioning and commissural axons in the zebrafish diencephalon

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As the brain develops in bilaterians neural connections that span the midline are established to ensure appropriate interhemispheric communication. Commissural neurons send axons across the midline in search of distant targets. In the zebrafish forebrain three Slit ligands and three Roundabout (Robo) receptors play major roles in the establishment of the astroglial growth substrate and direct guidance of commissural axons over that substrate. We previously demonstrated that manipulating Slit signaling affects positioning of these glia. Moreover we have shown that Slit1a behaves differently than known repellants, Slit2 and Slit3. Using gain and loss of function approaches we have characterized the relative contributions that the different Robo receptors exhibit in mediating Slit guidance for diencephalic astroglia and postoptic commissural axons. POC axons are seen to wander off of their preferred astroglial substrate to Slit1a misexpressing cells. Furthermore, we demonstrate that Robo1 and Robo3 mediate Slit2/3 repulsion of astroglia necessary to form condensed supportive glial bridges, while Robo2 and Robo3 are required for the direct response of POC axons to Slit2/3 surrounding repulsion. Our preliminary data suggests that Robo1